Objectives:
1. learn how to use a microscope to examine microbes
2. learn to recognize the characteristics of different microbes
3. practice formatting figures for the results section of a scientific paper and poster

Required Materials:
- immersion oil
- microscopes
- Kim-wipes
- prepared microscope slides
- lens paper

Procedure:
Before proceeding, you should read through and practice the procedures outlined in the document "Introduction to the Microscope."

1. Obtain one prepared microscope slide with a sample of bacteria. Many are available:
   - cocci
   - bacilli
   - *Spirillum rubrum*
   - bacillus, rod-shaped, negative stain
   - Gram positive cocci
   - Gram negative and positive organisms
   - *Neisseria gonorrhea*

Examine the bacterial cells using the oil immersion lens. Determine the shape and size of an average cell.

2. Obtain a prepared microscope slide with a sample of algae. Three different species are available:
   - *Cladophora* (green algae)
   - *Volvox*
   - *Spirogyra*

Examine the algal cells using the low power or high power lens. Determine the shape and size of an average cell.

3. Obtain a prepared microscope slide with a sample of fungi. Five different species are available:
   - *Aspergillus*
   - *Penicillium*
   - *Saccharomyces* (yeast)
   - *Rhizopus*
   - *Coprinus*

Examine the fungal samples using the low power lens; use the high power or oil immersion lens for the yeast sample. Describe your field of view. Estimate the size of the sample.

4. Use the procedure outlined in the protocol, "Staining with Methylene Blue," to examine one of the colonies growing on a plate from a previous lab. Use the digital camera to photograph the cells.

5. Use the procedure outlined in the protocol, "The Gram Stain," to examine one of the colonies growing on a plate from a previous lab. Use the digital camera to photograph the cells.

When you are finished:
- Use lens paper to wipe off the objectives and ocular lenses. (especially the oil lens)
- Place the scanning objective in the viewing position.
- Carefully wrap the electrical cord around the microscope, avoiding contact with the objectives.
- Cover the microscope with its protective cover and replace it in the microscope closet.
Staining with Methylene Blue

**Required Materials:**
- methylene blue
- microscope
- inoculating loop
- immersion oil
- wash bottles
- staining racks
- burners
- microscope slides
- bibulous paper
- sample

**Procedure for Bacteria Growing on Solid Media**

1. Using an inoculating loop, place a very small drop of tap water on a clean microscope slide.
2. Use a sterilized inoculating loop to pick up a small amount of one bacterial colony and spread it in the drop of water on the microscope slide.
3. Let dry at room temperature. DO NOT blow on the sample; DO NOT wave it in the air; DO NOT warm it to accelerate the drying step.
4. Once the smear has dried, grasp the slide with forceps and heat-fix the sample to the slide by briefly passing the slide through a flame.
5. Your sample is now ready for staining. Place your slide on a staining rack over the sink.
6. Flood the smear with methylene blue.
7. After two to three minutes, use a wash bottle to rinse the methylene blue from the slide. Do this so that the stain washes into the sink and not onto any other surfaces. Methylene blue may stain permanently.
8. Blot your slide dry with bibulous paper. Do not wipe the surface of the slide or you will remove your sample!
9. Examine your sample using a microscope. It will be necessary to use the 100X lens and immersion oil. Refer to the handout “Introduction to the Microscope” for advice on microscopy techniques.

**Procedure for Bacteria Growing in Liquid Media**

1. Use a sterilized inoculating loop to transfer a loopful of bacteria in liquid media onto a microscope slide.
2. Allow sample to air dry.
3. Heat fix and stain as for samples from solid media.
The Gram Stain

**Required Materials:**
- microscope
- microscope slides
- wash bottles
- inoculating loop
- burner
- crystal violet
- Gram's iodine
- alcohol
- bacterial sample
- mix of Gram (+) and Gram (-) bacteria

**Procedure:**

1. Prepare two samples on a single slide. Reserve one half of the slide for the positive and negative controls and the other half of the slide for your unknown sample.

2. Heat-fix a THIN smear of your sample microbe to half of a microscope slide. It is important to use a fresh sample because dead bacteria always stain Gram negative. (Briefly: mix microbe with water; spread thinly; air dry; quickly pass through flame)

3. Heat-fix a THIN smear of the mix of Gram positive and Gram negative controls to the other half the microscope slide.

4. Place the slide on a staining rack over a sink and cover smears with crystal violet. Allow the stain to react for 30 – 60 seconds. Note: All cells will stain Gram positive if you add stain while the slide is hot.

5. Rinse off the crystal violet with water. Use as little water as possible. You may use a wash bottle or water from a dropper bottle. Shake the slide to remove excess water.

6. Place slide on staining rack over sink and cover smears with Gram's iodine. Allow the stain to react for 30 – 60 seconds. Wash off the stain with water.

7. Hold the slide at a 45° angle over the sink and apply the alcohol decolorizer (95% EtOH) drop by drop to the slide and allow the alcohol to immediately fall into the sink. Stop when the rinse liquid is no longer colored. Immediately rinse the slide with water. Shake slide to remove excess water.

8. Place slide on staining rack over sink and cover smears with the counterstain safranin. Allow the stain to react for 1 – 2 minutes. Wash off the stain with water.

9. Blot the slide dry with bibulous paper before viewing with the microscope.

10. Determine if your unknown is Gram positive (purple) or Gram negative (pink).

**Notes:**
- If you over-colorize or under de-colorize – remove stain and immersion oil with alcohol and re-stain.
- Some non-bacteria with thick cell walls (e.g. some yeasts) also stain Gram positive.
Assignment: one per team – combined with “Collecting Microbes” lab assignment
due: September 17, 2008

Combine the results from your “Collecting Microbes” lab with the results from your “Microscopic Examination” lab.

Since the IA project requires production of a poster as well as a paper, the objective of this assignment is to familiarize you with the formatting requirements of both types of presentations. For advice on how to write the results section for a poster, refer to the handout, “Poster Presentations.” For advice on how to write the results section for a scientific paper, refer to the document, “How to Write a Scientific Paper.” General advice on formatting may be found on the website:
   http://web2.slc.qc.ca/adera/formatting.htm

Poster Presentation – Results section

1. Using the format appropriate for poster presentations, provide descriptions and a large image of the growth observed on one of the agar plates from the sections: Collecting microbes from small objects, Collecting microbes from large objects, or Collecting microbes from the air.

2. Using the format appropriate for poster presentations, provide a brief description and a large image of the growth observed on the m-HPC agar (heterotrophic count). Your description must specify the source of your water sample and an estimate of the number of bacteria present per milliliter of water.

3. Using the format appropriate for poster presentations:
   a. Provide a description and a photomicrograph image of unknown cells. Your description should include a description of the colony as well as the source of the sample.
   b. Use a table to compare the bacterial, fungal and algal cells that you examined under the microscope.

Scientific Paper – Results section

4. Using the format appropriate for scientific papers, provide descriptions and an image of the growth observed on another agar plate from the sections: Collecting microbes from small objects, Collecting microbes from large objects, or Collecting microbes from the air.

5. Using the format appropriate for scientific papers, provide a brief description and an image of the growth observed (if any) on the Levine-EMB agar (coliform count). Your description must specify the source of your water sample and an estimate of the number of coliforms present per milliliter of water.

6. Using the format appropriate for scientific papers:
   a. Provide a description and a photomicrograph image of unknown cells. Your description should include a description of the colony as well as the source of the sample.
   b. Use a narrative/text format to compare the bacterial, fungal and algal cells that you examined under the microscope.

7. Complete and hand in a peer evaluation for this lab assignment (lab work, poster, and paper)
Sample Marking Key – Poster Presentation Results Section

/6 Agar Plate
/4 description complete
   ___ sample/source
   ___ number/frequency
   ___ color
   ___ size
   ___ shape
   ___ margin
   ___ elevation
/2 image
   ___ elements of figure identified
   ___ size/total magnification indicated
   ___ figure large
   ___ good quality image

/6 Water Sample – mHPC plate
/3 brief description of colonies on plate
   ___ sample/source
   ___ number/frequency
   ___ color
   ___ size
/1 number of CFU per mL
/2 image
   ___ elements of figure identified
   ___ size/total magnification indicated
   ___ figure large
   ___ good quality image

/7 Unknown cell
/5 description
   ___ sample/source
   ___ colony characteristics
   ___ cell size
   ___ cell shape
   ___ Gram reaction
/2 image
   ___ elements of figure identified
   ___ size/total magnification indicated
   ___ figure large
   ___ good quality image

/6 Comparison of bacterial, fungal and algal cells
   similarities
   differences
   table formatted properly

/2 Formatting appropriate for poster
/3 Grammar and Style

/30 Total

Grammar and Style - Posters
   ___ reduce content
   ___ omit irrelevant details
   ___ increase font size
   ___ incorrect symbol
   ___ use ., not , to delineate decimal point
   ___ use Equation Editor
   ___ use citation format: (author, year)
   ___ italicize Latin names
   ___ use consistent formatting
   ___ simplify writing
   ___ use active voice
   ___ use past tense when describing your own work or ideas
   ___ avoid quotes
   ___ use °C symbol
   ___ add a space between number and units
   ___ lacks editing or proof-reading
   ___ see English Writing Workshop

Table Format – Posters
   ___ integrate into the text
   ___ provide informative title or heading
   ___ provide column headings
   ___ align numbers at decimal point
   ___ center data in column
   ___ omit vertical lines
   ___ minimize horizontal lines
   ___ describe data in table
   ___ single-space description

Figure Format - Illustrations and Photos – Posters
   ___ integrate into the text
   ___ provide informative title or heading
   ___ identify elements of figure
   ___ provide indication of size/total magnification
   ___ enlarge figure
   ___ improve figure quality or neatness
   ___ describe image
   ___ single-space description

Figure Format – Graphs – Posters
   ___ integrate into the text
   ___ provide informative title or heading
   ___ place independent var. on X axis. label.
   ___ place dependent var. on Y axis. label.
   ___ provide key to symbols/legend
   ___ enlarge
   ___ omit areas of graph without data
   ___ remove gray background
   ___ describe data in graph
   ___ single-space description
Sample Marking Key – Scientific Paper Results Section

/6 Agar Plate
/4 description complete
___ sample/source
___ number/frequency
___ color
___ size
___ shape
___ margin
___ elevation
/2 image
___ elements of figure identified
___ size/total magnification indicated
___ figure large
___ good quality image

/6 Water Sample – Levine-EMB plate
/3 brief description of colonies on plate
___ sample/source
___ number/frequency
___ color
___ size
/1 number of coliforms per mL
/2 image (if colonies present on plate)
___ elements of figure identified
___ size/total magnification indicated
___ figure large
___ good quality image

Unknown cell
/5 description
___ sample/source
___ colony characteristics
___ cell size
___ cell shape
___ Gram reaction
/2 image
___ elements of figure identified
___ size/total magnification indicated
___ figure large
___ good quality image

/6 Comparison of bacterial, fungal and algal cells
similarity
differences
comparison made

/2 Formatting appropriate for scientific paper

/3 Grammar and Style

/30 Total

Grammar and Style – Scientific Paper
___ use past tense when describing your own work or ideas
___ use double or 1½ spacing
___ provide page numbers
___ use citation format: (author, year)
___ simplify writing
___ use active voice
___ avoid quotes
___ italicize Latin names
___ use °C symbol
___ add a space between number and units
___ incorrect symbol
___ use Equation Editor
___ use consistent formatting
___ lacks editing or proof-reading
___ see English Writing Workshop or peer tutor

Table Format – Scientific Paper
___ provide table number
___ provide informative title
___ place number and title above table
___ make table independent
___ provide column headings
___ align numbers at decimal point
___ center data in column
___ omit vertical lines
___ minimize horizontal lines
___ make table independent
___ provide descriptive text if needed

Figure Format – Illustrations and Photos – Scientific Paper
___ provide figure number
___ provide informative title
___ place number and title below figure
___ identify elements of figure
___ provide indication of size/total magnification
___ enlarge figure
___ improve figure quality or neatness
___ make figure independent
___ describe image
___ single-space description

Figure Format – Graphs – Scientific Paper
___ provide figure number
___ provide informative title
___ place number and title below figure
___ place independent var. on X axis. label.
___ place dependent var. on Y axis. label.
___ provide key to symbols/legend
___ enlarge
___ omit areas of graph without data
___ remove gray background
___ make figure independent
___ describe data in graph
___ single-space description

Note: Avoid beginning sentences with “The table” or “The figure” which makes the table (or figure) the topic of the sentence. Instead, make the data within the table or figure the subject of sentences.